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September 4, 1991

DATA EVALUATION RECORD

CHLOROTHALONIL

Metabolism in Rats

STUDY IDENTIFICATION: Savides, M.C., Marciniszyn, J.P., and Killeen, J.C., Jr. Study to determine the metabolic pathway for chlorothalonil following dermal application to rats. (Unpublished study No. 1625-87-0057-AM-001 performed by Ricerca, Inc., Painesville, OH, for Fermenta ASC Corp., Mentor, OH; dated May 5, 1989.) MRID No. 412505-08.

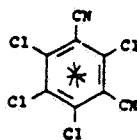
APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
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Signature: *Robert J. Weir*

Date: Sept. 4, 1991

1. CHEMICAL: Terachloroisophthalonitrile; 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile; 1,3-dicyano-2,4,5,6-tetrachlorobenzene; chlorothalonil.
2. TEST MATERIAL: [¹⁴C]Chlorothalonil, uniformly labeled in the benzene ring, and unlabeled chlorothalonil were used. The radiolabeled test material had a radiochemical purity of 97.6 percent (specific activity of original test material not reported). The unlabeled test material had a chemical purity of 95.4 percent. The structure and radiolabel (*) position of [¹⁴C]chlorothalonil are shown below:



3. STUDY/ACTION TYPE: Metabolism in rats following dermal application.
4. STUDY IDENTIFICATION: Savides, M.C., Marciniszyn, J.P., and Killeen, J.C., Jr. Study to determine the metabolic pathway for chlorothalonil following dermal application to rats. (Unpublished study No. 1625-87-0057-AM-001 performed by Ricerca, Inc., Painesville, OH, for Fermenta ASC Corp., Mentor, OH; dated May 5, 1989.) MRID No. 412505-08.
5. REVIEWED BY:

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7. CONCLUSIONS:

- A. Small amounts (mean 3.11 percent; range 2.52 to 4.00 percent) of a dermal dose of [¹⁴C]chlorothalonil (equivalent to 5 mg/kg) were absorbed by male rats (five/group; four groups) during a 48-hour exposure period. About half of the ¹⁴C absorbed (1.5 percent of the administered dose) was recovered from urine samples collected during the first 24 hours of exposure to the compound; the remaining 1.6 percent was recovered from 24- to 48-hour samples. The nature and amounts of urinary thiol metabolites of chlorothalonil varied quantitatively and qualitatively. The total amount of urinary thiol metabolites was low for all groups and varied considerably, accounting for approximately 0.04 to 2.7 percent of the total urinary radioactivity and 0.002 to 0.07 percent of the administered radioactivity. Detectable amounts of a tri-thiol metabolite of chlorothalonil were present in all groups of rats; in contrast, a di-thiol metabolite was present in the urine of two of the four groups, and the urine of only one group of rats contained mono-, di-, and tri-thiol metabolites. Differences in the detection of metabolites were most likely due to insufficient amounts of radiolabeled material in the urine.
- B. This study provides supplementary information on the metabolism and elimination of dermally administered [¹⁴C]chlorothalonil in male rats. Reproducibility of results was poor; specifically, the quantitative and qualitative recovery of urinary thiol metabolites varied considerably among similarly dosed animals. In addition, a rationale for using only males rats in this study was not provided.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

- 1) [¹⁴C]Chlorothalonil (lot No. not reported) was 97.6 percent pure, as determined by reverse-phase high-performance liquid chromatography (HPLC). The non-labeled, analytical grade test material (lot No. not specified) had a chemical purity of 95.4 percent, as determined by gas chromatography (GC). Impurities were not identified for either test material.

¹Only the items appropriate to this DER have been included.

- 2) Male CD Sprague-Dawley rats (Charles River Laboratories, Portage, MI), weighing between 211 and 267 g at the time of dosing, were used. Animals were quarantined for at least 7 days before application of the test material.
- 3) The dosing solution was prepared by diluting a stock solution of [^{14}C]chlorothalonil with nonlabeled chlorothalonil to the appropriate specific activity. The test material in solution was then divided equally among four vials, the solvent was evaporated, and the vials were frozen and stored until use.

The stock solution of radiolabeled chlorothalonil was prepared by dissolving 8.35 mg [^{14}C]chlorothalonil in 10 mL methylene chloride. A 0.01-mL aliquot of this solution was then diluted with methanol to a final volume of 10 mL and analyzed for radiochemical purity by HPLC and liquid scintillation counting (LSC). The purity and specific activity of the [^{14}C]chlorothalonil in the methanol-diluted stock solution were 98.7 percent and 120.5 mCi/mmol, respectively.

Unlabeled chlorothalonil (28.67 mg) was added to the stock solution of radiolabeled test material to give a final specific activity of 27.82 mCi/mmol (as determined by GC and LSC), and the solution was divided among four vials. The contents of the vials were evaporated to dryness under nitrogen and stored in the dark at -70°C until use.

Immediately prior to dosing, 6 mL of acetone was added to a vial (giving a chlorothalonil concentration of 1.58 mg/mL), and the appropriate dosing volume for each animal in a group was determined based on body weight and the specific activity and chlorothalonil concentration of the stored material.

- 4) Four groups (groups A, B, C, and D) of male rats (five/group) received a nominal dose of 5 mg [^{14}C]chlorothalonil/kg (target, 103 to 131 μCi /animal); the compound was administered in a single dermal application of approximately 1.5 mg test material/mL over an average skin area of 25 cm^2 .

At 24 hours before dermal application of [^{14}C]chlorothalonil, the dorsal hair (approximately 40 cm^2) of each rat was clipped. Animals were then placed in individual metabolism cages, and predosing urine samples were collected. Immediately before dosing, animals were weighed, and dosing volumes were calculated. Rats were anesthetized with ether, and the

calculated dosing volume was applied with a syringe to a 25-cm² area of the clipped skin. The treated area was covered with a nonocclusive patch, and a neoprene rubber template/wire mesh screen device was glued to the back of the animal to prevent loss of the test material. Rats were exposed dermally to the [¹⁴C]chlorothalonil solution for 48 hours; urine and feces were collected separately over dry ice at 24 and 48 hours postapplication.

- 5) Thiol metabolites in the urine were isolated by extraction of the urine with ethyl acetate and methylation of the extracts followed by column chromatography. The fractions corresponding to the thiol metabolites for groups A and B were analyzed by gas chromatography (GC)/mass spectroscopy (MS); those from groups C and D were analyzed by GC/selective ion monitoring (SIM).

The volume and pH of urine samples were determined, and aliquots were taken for LSC. Urine samples were filtered and pooled according to dosing group and collection period; aliquots were acidified to pH 2.0 and extracted three times with acidified ethyl acetate. Extractable organic fractions were reduced to dryness, methylated twice with diazomethane, and analyzed by reverse-phase HPLC/LSC. Aliquots of pooled 0- to 24-hour and 24- to 48-hour urine samples from all groups, and aliquots of the nonextractable aqueous phase and the methylated organic phase, were analyzed by reverse-phase HPLC/LSC.

For isolation and identification of the thiols for groups A and B, the methylated fractions from each group were pooled (0 to 48 hours) and placed on a flurasil column. The column was washed four times with hexane to remove interfering substances, eluted with ethyl ether:hexane (20:80 v/v) to elute thiols, and washed with methanol. The ether/hexane was evaporated, and florisil chromatography was repeated, followed by repeated cycles of HPLC and florisil chromatography of isolated peaks. The methanol fractions for group B were also concentrated in an attempt to recover additional radioactive material. Samples of interest were analyzed by GC/MS.

Individual derivatized samples (0 to 24 hours and 24 to 48 hours) from groups C and D were chromatographed by reverse-phase HPLC. The radioactive peaks that corresponded in elution time to methylated thiol standards of chlorothalonil thiol metabolites were collected, concentrated to dryness, and extracted with

ethyl ether and ethyl acetate. These extracts were then concentrated by drying under nitrogen and reconstituted in either ethyl ether or ethyl acetate, depending on solubility. The reconstituted samples were analyzed by GC/SIM.

The limit of detection for samples analyzed by GC/MS was 50 ng; the sensitivity limit for samples analyzed by GC/SIM was 3 ng. The molecular ions monitored were the methylated mono-, di-, and tri-thiol metabolites of chlorothalonil.

No fecal samples were analyzed.

- B. Protocol: The protocol and protocol amendments for this study are presented in the appendix.

12. REPORTED RESULTS:

- A. Rats in all groups received an average (\pm standard deviation) of 4.6 ± 0.3 mg [^{14}C]chlorothalonil/kg (range 4.38 to 4.99 mg/kg). The average amount of radiolabel administered to each animal was 115.4 ± 7.0 μCi (range 106.5 to 123.25 μCi). A description of how the actual doses were determined was not provided.
- B. Animals in all groups excreted approximately 3.11 percent (range 2.52 to 4.00 percent) of the ^{14}C dose in the urine during the 48 hours after compound application (Table 1). About half (1.5 percent of the ^{14}C dose) was recovered from samples collected during the first 24 hours; the remaining 1.6 percent was recovered from the 24- to 48-hour urine samples.
- C. For groups A and B, total methylated thiols accounted for 2.74 and 0.26 percent of the total urinary radioactivity, respectively (Table 2); these values corresponded to 0.07 and 0.01 percent of the administered ^{14}C dose. Animals in group A excreted both di- and tri-thiols (1.58 and 1.16 percent of the urinary ^{14}C , respectively), but animals in group B excreted only the tri-thiol analog of chlorothalonil. Because of the large variability in the recovery of methylated thiols between groups A and B, the experiment was repeated (groups C and D) and the methodology for isolating thiol metabolites was modified.

In groups C and D, total thiols accounted for approximately 0.33 to 0.45 and 0.0376 to 0.0493 percent of the total urinary ^{14}C eliminated by rats, respectively (Table 2). The maximum amount of thiols recovered from the urine of these

TABLE 1. Percent Recoveries of Radioactivity in the Urine of Rats Exposed Dermally to [^{14}C]Chlorothalonil for 48 Hours^a

Group	Percent of ^{14}C administered:		
	0-24 hr ^b	24-48 hr	0-48 hr
A	1.22 ^c	1.30	2.52
B	1.45	1.74	3.19
C	1.34	1.38	2.72
D	2.10	1.90	4.00
Mean (\pm S.D.)	1.53 \pm 0.39	1.58 \pm 0.29	3.11 \pm 0.66

^aAnimals received an average dose of 4.6 mg [^{14}C]chlorothalonil/kg.

^bUrine collection times.

^cEach value is the mean of five animals.

Source: CBI p. 27.

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TABLE 2. Distribution of Thiols in the Urine of Rats Exposed Dermally to 5 mg [¹⁴C]Chlorothalonil/kg for 48 Hours

Group ^a	Time Period	Mono-SH ^b	Di-SH ^b	Tri-SH ^b	Total SH ^c
A	0-48 hr	ND ^d	1.5818	1.1600	2.7418
B	0-48 hr	ND	ND	0.2624	0.2624
C	0-24 hr	0.0055-0.0076	0.0092-0.0142	0.0422-0.0554	0.0569-0.0772
	24-48 hr	ND	0.0697-0.1074	0.2034-0.2622	0.2731-0.3696
	Total				0.3300-0.4468
D	0-24 hr	ND	ND	0.0203-0.0266	0.0203-0.0266
	24-48 hr	ND	ND	0.0173-0.0227	0.0173-0.0227
	Total				0.0376-0.0493

^aFor groups A and B, quantitation was by GC/MS and for Groups C and D quantitation was by GC/SIM. Each group consisted of five male rats.

^bAnalyzed and quantitated as methylated thiols. Reported as a percent of the total radiolabel in urine.

^cTotal thiols are the sum of the individual thiols in each group.

^dNot detected (3 ng sensitivity limit by GC/SIM; 50 ng limit of detection by GC/MS).

Source: CBI p. 29.

rats, as reported by the study authors, represented 0.007 percent of the radioactivity administered to animals in group C and 0.001 percent of that applied to rats in group D. These values were recalculated by the reviewers to be 0.012 and 0.002 percent of the administered ¹⁴C dose, respectively. See Reviewers' Discussion of this DER for additional details.) The tri-thiol was identified in all urine samples from groups C and D. The di-thiol was recovered from the 0- to 24-hour and 24- to 48-hour urine samples of animals in group C; the group D urine samples did not contain this metabolite. The mono-thiol metabolite of chlorothalonil was present only in the 0- to 24-hour urine of group C rats.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

- A. Rats in each dose group eliminated approximately 1.5 percent of the ¹⁴C dose in the urine during the first 24 hours after dosing and an additional 1.6 percent in the urine collected between 24 and 48 hours postapplication. The GC/MS and GC/SIM analyses of urine indicated that a maximum of only 0.07 percent of the administered dose was excreted as thiol metabolites. In contrast, urine from rats orally administered ¹⁴C-chlorothalonil (5 mg/kg/day for 5 days) contained an estimated 1.6 percent of the administered dose and approximately 20 percent of the urinary radiolabel as thiol metabolites (data presented in a footnote on CBI pp. 32 and 33; oral dosing data from SDS Biotech Corporation unpublished study No. 1173-84-0079-AM-003). This represents a 20-fold difference between the oral and dermal exposure groups with respect to the amounts of thiols excreted following the same dose (5 mg chlorothalonil/kg). The study authors noted that the glutathione pathway is involved in the metabolism of chlorothalonil to thiol metabolites and that the thiols generated may be responsible for the nephrotoxicity observed in animals exposed to this fungicide. They concluded that because excretion of these potentially nephrotoxic thiols was much less in the dermally exposed rats than in those exposed orally, dermal exposure may be associated with a lower risk of toxicity as compared with oral exposure (given the same dosage in mg/kg).

The GC/MS results for groups A and B also indicated a large discrepancy in the amounts of thiol metabolites recovered from the urine of rats in these two groups. For group A animals, total thiol metabolites accounted for 2.74 percent of the urinary radioactivity and 0.07 percent of the ¹⁴C dose; in contrast, thiol metabolites of chlorothalonil accounted for only 0.26 percent of the urinary ¹⁴C and 0.01 percent of the ¹⁴C dose in animals of group B. In addition,

both the di- and tri-thiol metabolites were recovered from the urine of group A rats, accounting for approximately 1.58 and 1.16 percent of the urinary radioactivity, respectively, whereas only the tri-thiol metabolite was identified in the urine of rats in group B.

In an attempt to reconcile these differences, the study authors repeated the dermal exposure study using an additional 10 male rats (groups C and D). However, this repeated experiment did not yield markedly improved results (total thiols represented up to 0.45 and 0.049 percent of the urinary ^{14}C of group C and D rats, respectively), and the study authors provided no explanation for the poor reproducibility among the four groups. The total amounts of thiols excreted by rats from groups B and C were comparable (i.e., 0.26 and 0.45 percent of the urinary ^{14}C , respectively), but results from groups A and D were not in agreement with each other or with group B or C. It was suggested that the amount of thiols excreted in the urine of animals in group A (i.e., 2.74 percent of the total urinary ^{14}C) was probably high.

The study authors concluded that the urinary metabolite profiles of animals in the four exposure groups differed quantitatively but not qualitatively. They noted that the tri-thiol analog of chlorothalonil was the major urinary metabolite excreted by dermally exposed rats.

- B. A quality assurance statement and a statement of compliance with Good Laboratory Practices, both signed and dated May 8, 1989, were included in the report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This study provides only supplementary information on the metabolism and elimination of dermally administered [^{14}C]chlorothalonil in male rats. A major problem with this study involved the poor reproducibility and wide variability of the results, which made it difficult to determine how much of a 5-mg/kg dermal dose of [^{14}C]chlorothalonil was eliminated, on an average, as thiol metabolites, and to evaluate the relative importance of thiol and glutathione metabolism of the fungicide following dermal exposure. In addition, values in the summary tables in the CBI could not be verified from the raw data. Another deficiency in this study, per EPA guidelines (85-1), was the use of only male rats; the study authors did not explain why female rats were excluded. The reviewers assumed that the urine and feces were collected until sacrifice (as stated in the protocol, CBI p. 54), rather than during the 48 hours after the 48-hour dermal exposure period, as is implied in the experimental section of the report (CBI p. 22).

Urinary excretion data indicated that only a small amount (mean 3.11 percent; range 2.52 to 4.00 percent) of the dermal dose of [¹⁴C]chlorothalonil was absorbed during the 48-hour exposure period. Information on the amount of ¹⁴C excreted in the feces and remaining at the application site would have supported and complemented these data. Urinary elimination of ¹⁴C appeared to be lower in dermally exposed rats than in orally dosed rats, indicating reduced absorption of the fungicide by the former route of exposure.

The total amount of thiol metabolites in the urine was low for all groups and varied considerably, accounting for approximately 0.04 to 2.7 percent of the total urinary radioactivity and 0.002 (as calculated by the reviewers) to 0.07 percent of the administered radioactivity. The maximum percent of the administered dose excreted as thiols appears to have been miscalculated by the study authors for groups C and D. They reported that 0.007 and 0.001 percent of the ¹⁴C dose was excreted as thiols, respectively (CBI p. 30); however, when recalculated by the reviewers, these values corresponded to approximately 0.012 [0.004468 (the fraction (percent = 0.4468) of the urinary radiolabel excreted as thiol metabolites) \times 2.72 percent (percent of administered dose excreted in the urine)] and 0.002 (0.000493×4.00) percent of the ¹⁴C dose administered to rats in groups C and D, respectively.

The urinary excretion patterns of thiol metabolites also varied qualitatively: animals in groups B and C excreted detectable amounts of only the tri-thiol metabolite; both the di- and tri-thiol compounds were detected in group A samples; and all three methylated thiol compounds (mono-, di-, and tri-) were excreted by animals in group C. Differences in the detection of metabolites were most likely due to insufficient amounts of radiolabeled material, although samples from groups C and D were analyzed by GC/SIM at a detection level of 3 ng. Extraction, isolation, and cleanup procedures appeared to be appropriate, but repeated chromatographic analyses/extractions resulted in losses and reduced the amount of radioactivity available for metabolite identification. The tri-thiol metabolite was the principal urinary thiol metabolite, representing approximately 70 to 100 percent of the total thiols excreted by rats in groups B, C, and D, and 42 percent of that eliminated by group A rats. Glutathione and mercapturic acid conjugates of chlorothalonil and other metabolites were not identified.

Although direct comparisons between the oral dosing data presented by the study authors and the results of the dermal exposure experiments are not appropriate, the suggestion that rats administered oral doses of chlorothalonil eliminate much larger amounts of thiols in the urine (as percent of total

urinary metabolites and percent of dose) than dermally exposed rats appears acceptable.

Items 15 and 16--see footnote 1.

APPENDIX

Protocol and Protocol Amendments
(CBI pp. 40-65)

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EP

RICERCA, INC.
DEPARTMENT OF TOXICOLOGY AND
ANIMAL METABOLISM

STUDY TO DETERMINE THE METABOLIC
PATHWAY FOR CHLOROTHALONIL FOLLOWING
DERMAL APPLICATION TO RATS

PROTOCOL
DOCUMENT NUMBER: 1625-87-0057-AM-000
SDS-2787

Ricerca, Inc.
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Unit Document Reference Number: 87-0057

RIN 8587-93

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Pages 18 through 40 are not included.

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